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A drug dependent proliferative switch for genetically modified cells

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A versatile synthetic dimerizer for the regulation of protein-protein interactions

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ABSTRACT: The use of low molecular weight organic compounds to induce dimerization or oligomerization of engineered proteins has wide-ranging utility in biological research as well as in gene and cell therapies. Chemically induced dimerization can be used to activate intracellular signal transduction pathways or to control the activity of a bipartite transcription factor. Dimerizer systems based on the natural products cyclosporin, FK506, rapamycin, and coumermycin have been described. However, owing to the complexity of these compounds, adjusting their binding or pharmacological properties by chemical modification is difficult. We have investigated several families of readily prepared, totally synthetic, cell-permeable dimerizers composed of ligands for human FKBP12. These molecules have significantly reduced complexity and greater adaptability than natural product dimers. We report here the efficacies of several of these new synthetic compounds in regulating two types of protein dimerization events inside engineered cells-induction of apoptosis through dimerization of engineered Fas proteins and regulation of transcription through dimerization of transcription factor fusion proteins. One dimerizer in particular, AP1510, proved to be exceptionally potent and versatile in all experimental contexts tested.

Functional analysis of Fas signaling in vivo using synthetic inducers of dimerization

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ABSTRACT: Background: Genetic abnormalities in the Fas receptor or its trimeric ligand, FasL, result in massive T-cell proliferation and a lupus-like autoimmune syndrome, which was initially attributed to excessive lymphoproliferation but is now ascribed to the absence of Fas-mediated cell death. Although Fas is normally expressed on most thymocytes, negative selection seems to be unperturbed in Fas-deficient (lpr) mice. This suggests that Fas has an important function in peripheral, but not thymic, T cells. Results: To explore the Fas-mediated cell death pathway both in vitro and in vivo, we used conditional alleles of the Fas receptor that can be triggered by an intracellularly active chemical inducer of dimerization known as FK1012. We found that membrane attachment is important for Fas function and, unlike previous results with anti-Fas monoclonal antibodies, we show that dimerization is sufficient to trigger apoptosis. Finally, the administration of FK1012 in vivo to transgenic animals expressing the conditional Fas receptor in thymocytes demonstrates that sensitivity to Fas-mediated apoptosis is restricted to CD4⁺ CDB⁺ thymocytes. Conclusions: Here, we describe the first in vivo application of non-toxic, cell-permeable synthetic ligands to regulate signal transduction in transgenic mice expressing a conditional receptor. Using this system, we show that the Fas pathway is restricted to double-positive thymocytes in vivo, consistent with recent in vitro findings with thymocytes. This method promises to be useful not only for developmental studies involving cell ablation, but also for studies involving the regulation of a wide variety of signaling molecules.

Controlling signal transduction with synthetic ligands.

Spencer, David M.; Wandless, Thomas J.; Schreiber, Stuart L.; Crabtree, Gerald R.

Science, v262, n5136, p1019(6)

Nov 12,

1993

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AUTHOR ABSTRACT: Dimerization and oligomerization are general biological control mechanisms contributing to the activation of cell membrane receptors, transcription factors, vesicle fusion proteins, and other classes of intra- and extracellular proteins. Cell permeable, synthetic ligands were devised that can be used to control the intracellular oligomerization of specific proteins. To demonstrate their utility, these ligands were used to induce intra-cellular oligomerization of cell surface receptors that lacked their transmembrane and extracellular regions but contained intracellular signaling domains. Addition of these ligands to cells in culture resulted in signal transmission and specific target gene activation. Monomeric forms of the ligands blocked the pathway. This method of ligand-regulated activation and termination of signaling pathways has the potential to be applied wherever precise control of a signal transduction pathway is desired.

Mechanistic studies of a signaling pathway activated by the organic dimerizer FK1012.

Pruschy M N; Spencer D M; Kapoor T M; Miyake H; Crabtree G R; Schreiber S L

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BACKGROUND: The T-cell receptor (TCR) signaling pathway is initiated by regulated association of TCR chains, including the zeta chain. A recently reported method for inducing the dimerization or oligomerization of targeted proteins in cells used the TCR pathway as a test system. In cells transfected with cDNA encoding MZF3E, a chimeric receptor comprising the intracellular domain of the zeta chain and three copies of FK506-binding protein (FKBP), low concentrations of a synthetic dimer of the natural product FK506 (FK1012) activated the expression of reporter genes. We set out to examine the signaling pathway initiated by FK1012. RESULTS: We characterized the effect of FK1012 on MZF3E and a second chimeric receptor, MZF1E, which contains the zeta chain and one copy of FKBP. Only MZF3E gave FK1012-activated signaling, as shown by an increase in the kinase activity associating with MZF3E, and the appearance of specific phosphotyrosine-containing proteins. Signaling required localization of MZF3E to the inner plasma membrane, and activation of gene transcription in response to FK1012 was dependent on the protein phosphatase calcineurin and the transcriptional activator NF-AT. Some signaling events in the pathway had different kinetics when activated by MZF3E instead of the TCR, however. An unexpected requirement for the prolonged activation of calcineurin was observed. CONCLUSIONS: Synthetic dimerizers can be used to gain control over cellular processes that require the association of specific intracellular proteins. The TCR signaling pathway was selected as an initial test system; we show here that one can indeed activate this signaling pathway by inducing the oligomerization of the cytoplasmic tail of the zeta chain with the cell-permeable reagent FK1012.

Set	Items	Description
S1	400	E3-E17
S2	10	S1 AND FKBP
S3	5	RD (unique items)
S4	390	S1 NOT S2
S5	129	RD (unique items)
S6	43	S5 NOT PY>1997
S7	31	((DIMERIZ?)(3N) (RECEPTOR?)) (S) (FKBP OR FK506 OR FK1012)
S8	12	RD (unique items)
S9	3	S8 NOT PY>1997
S10	264	SYNTHETIC (2N) DIMERIZ?
S11	55	S10 AND FK?
S12	19	RD (unique items)
S13	4	S12 NOT PY>1997
S14	4	S13 NOT S3